HISTOLOGICAL STUDY ON A NOVEL BONE GRAFT SUBSTITUTE: HUMAN DERIVED TOOTH-HYDROXYAPATITE COMPARED WITH CORALLINE HYDROXYAPATITE

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Abstract-**Different types of bone-graft substitutes have been developed, and are on the market worldwide to eliminate the drawbacks of autogenous grafting. They vary in composition, strength, osteoinductive and osteoconductive properties, mechanism and rates at which they are resorbed and remodelled. Of these, THA is a novel material produced by one of us (FNO). This study was performed to determine the histological properties of THA on animals, and to achieve this a standard on the market coralline HA (CHA) was used as control. 20 sheep were used in this study and divided into 2 groups. Human THA (Group-A) and CHA (Group-B) materials were implanted to the tibiae of 10 sheep in each group. The histological examinations of surrounding bone response of the implant materials were done 12 weeks after implantation. There was no significant difference histologically between groups A and B. All materials were found to be surrounded by new bone tissue. THA was found to be as efficient as standard CHA on histological basis. In addition, economical production of THA should be taken into consideration. In future, THA may be a viable alternative on bone grafting when clinical trials were completed.**

***Key words*- Hydroxyapatite, grafting, bone graft, osteoconduction**

I. INTRODUCTION

Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH_2)$] is the main mineral constituent of human bone, and also an outstanding bone substitute because of its osteoconductive properties. HA ceramics can be manufactured from natural materials such as coral or bone [1]. Autologous bone grafting is still the clinical standard in the treatment of bone defects at present [2]. Autogenous bone grafts, are most common type of grafting material used in orthopaedic and reconstructive operations [3] because of minimal ethical or immunological concerns [4]. Main disadvantage of this operation is that it causes local bone defect at the donor site [3] (second site morbidity), limited availability, anatomical and structural problems and tendency towards resorption [4]. Allogeneic tissue bears the risk of infection and immune response [1]. There are also some concerns about requirement for immunosuppressant drugs, transmission of viral or prion contamination, ethical and religious concerns [4]. Allogeneic bone materials are resorbed more rapidly than autogenous bone due to immunogenetic responses [5]. Nevertheless, due to both availability and patient-associated problems, alternative treatment techniques are under development. The rationale of these new strategies is the construction of so-called bone graft subsitute [2]. Novel ceramics such as hydroxyapatite, bioglasses, bioactive glass ceramics and other calcium phosphates have shown to enhance bone formation [6]. Osseointegration is the procedure by which mature bone deposited directly on implant material without any intervening soft or fibrous tissue [7]. The extent of osseointegration between bone and newly implanted material is influenced by many factors like biomaterials topography and chemistry [6]. In osteoconduction, the graft material acts as a scaffold for deposition of new bone by creeping substitution from adjacent living bone. HA has been used for hard tissue replacement and augmentation due its biocompatibility and osteoconductive potentials. Because of the compositional similarity to hard tissues, such implants may be superior to alternative materials. Associated with its biocompatibility, there is interest in HA for applications involving orthopaedics and dentistry [8]. Coralline is known to be more soluble than HA. However, the larger size of the crystals, the existence of crystal bridges, the smaller surface area and the higher crystallinity of the ceramic when compared to bone, makes its remodelling substantially slower that of natural bone [1].

II. METHODOLOGY

In this study, HA prepared from human tooth (THA) and coralline HA (Pro Osteon 200, Interpore Cross, USA) (CHA) was used. THA material was derived from

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extracted, fresh human teeth. It was irrigated with tap water and soaked in a 1% concentration of an antiseptic solution to prevent bad odor and contamination of various infectious diseases. Subsequently the teeth were reirrigated and deproteinized in an alkali solution (1% concentration of sodium hypochloride). Thereafter samples were reirrigated with tap water again. All samples were calcinated to 5?C min-1 to 850?C for 2-3 hours. It was observed that at high temperatures the dentine matter and enamel matter separated easily and that nearly 60% of the material was dentine and 40% enamel. Dentine matter was grinded in an alumina mortar and sieved 100-150 ?m particle size [9-10].

Prior to this study, National Institute of Health (NIH) guidelines for the care and use of laboratory animals have been observed for feeding and anaesthetic procedures. 20 female sheep (each about 50-60 kg) were used in this study. HA and coral materials were sterilized in an autoclave. The sheep were operated under general anaesthesia with Rompun (Xylazine hydrachloride, Bayer, Ýstanbul, Turkey) and Ketalar (Ketamin HCl, Eczacýbaþý, Ýstanbul, Turkey). After anesthesia the tibial section of the right leg was shaved and disinfected. Under sterile surgical conditions after incising the skin, the muscles and periost were dissected. The spongious bone medially at the proximal metaphysis of the tibia was drilled with a 5 mm diameter bit, 1 cm depth. THA particles were placed into the drill hole. Then the layers were closed in the usual manner and skin was sutured. The same procedure was Performed for the left leg and CHA was applied for control purposes. Postoperatively the sheep were fed with rich libitum diet. 6 months after the operation the sheep were sacrificed and histopathological studies were performed. Histopathological specimens were prepared for light microscopic examination. Materials were fixed with a formalin-alcohol-acetic acid solution (TBD-2, Shandon Lipshaw, USA) for 18 hours, rinsed in 70 % alcohol and decalcified in commercial decalcifying solution for 4 days, and then embedded in paraplast. The 5-8 ?m thick sections were stained with hematoxylin-eosin (HE) and giemsa and examined with light microscopy.

There wasn't any postoperative infection among the sheep. THA and CHA particles were not rejected as foreign material and no sinus formation are wound complications were encountered.

III. Results

In the light microscopic examination particles of CHA and of THA were tolerated well and showed no evidence of infection, fibrosis or necrosis. Bone marrow elements around the implanted materials had a normal histological appearance. In those cases, both particles of DHA and THA were surrounded almost totally trabecular bone tissue (Figures 1 and 3). Polarization microscopy viewed organized arrangement of collagen fibre lameller pattern in a newly formed trabecular bone tissue around implanted particles (Figures 2 and 4). Only, minimal osteoclastic resorption was seen around implanted particles in which were implanted in subperiosteal field erroneously (or "particles escaped in subperiosteal field'').

IV. Discussion

Large bone defects, such as those encountered during total hip arthroplasty revision operations, after bone tumor removal and resulting from high-energy trauma are usually reconstructed with autogenous or allogenetic bone grafts. However, the supply of autogenous bone is limited and the procedure for harvesting bone grafting may itself result in considerable morbidity [5]. HA production by synthetic procedures has been found very costly [11]. It has been known long that abattoir-derived animal bone waste constitutes a potentially useful source of materials suitable for biomedical purposes. These materials can be produced by milling of defatted/solvent cleaned/boiled/bleached bone or from acid-digestion/sodium hydroxide (NaOH) reprecipitation of bone materials [12]. However, many investigators pointed the risk of using those implant materials about possible transmission of viruses and prions [5, 4, 13,1]. Zhang et al used demineralized bone matrixes (DBM) by exposure of ground bone (human originated) with dilute hydrochloric acid, which enables controlled demineralization of bone [14-15]. Many researchers also used demineralized bovine bone in their studies [6]. Lin et al. prepared sintered HA materials from bovine bone after boiling and dehydration in alcohol at 800?C temperature for 6 hours. Crystallographic investigations of the bone minerals have shown that, apart from the main fraction exhibiting the base of HA (containing stoichiometric HA and non-stoichiometric HA). It is known that bone contains other crystalline substances such as octacalcium phosphate, brushite and tricalcium phosphate (TCP). Mittelmeier heated the bovine bone and found that sintered bovine bone contained 93% of HA (natural bone contains 90 %) and 7 % TCP [13].

There are not much data about calcinating or ashing methods at high temperatures on literature. Vargas et al. used variable thermal processing temperatures between 300-1500?C. The authors stated that 500?C temperature had ?1 wt % and at 800?C temperature ?0.14 wt % residual carbon [17]. We had used 850?C sintering temperature in our study. In a recent study, Pongkao et al. prepared HA with calcinating method from cattle bone at 700?C in air for 3 hours. They had reported that the Ca: P ratio was 1.66 (heavy metals such as; As ?0.5 ppm, Cd

?0.5 ppm, Pb ?5 ppm, Hg ?1 ppm) [18]. Guizzardi et al. used ashing method at 400?C and also deproteinized the samples at sodium hypochloride. They had claimed that very high temperatures would destroy the bone architecture [19]. Ohbayashi et al. stated that HA specimens could induce osteogenesis if waited long enough such as 150-200 days even within muscle tissue without using bone morphogenetic protein. [3]. Broz et al. had also used ashing method. First they used sodium hypochloride bovine (NaOCl) solution to remove the collagen phase from cortical bone. In a report by Otter et al., by using NaOCl they were able to remove 99% of the collagen matrix. Güzelsu and Walsh had also used this method. Broz et al. had ashed the treated bone at 800?C for remainder proteins. They had found from their experiment that ashed bone demonstrates less induced damage than immersed bone. The disadvantage of ashing is that, the ashed bone was found to be more brittle [20]. This brittleness plays no specific role in our study because we had used our ashed HA-material in a granule form.

V. Conclusion

All of the recent researchers had used DBM, DHM (demineralized human matrix), ashed or calcinated HA from natural sources and many different calcium phosphates from reagent materials successfully in their studies. Nowadays the question comes about how safe would be these materials during grafting surgery. Life threatening diseases like Kreuz Jacopson, AIDS and many others will limit or at least shade the success of bone-graft materials. The use of ashed or calcinated natural bioceramics from natural sources will become more and more important. In our study we had seen from the histological studies that THA was very successful at surgery promoting osseointegration. THA seems to be a safe bone-graft bioceramic material for grafting, especially in orthopedic surgery. In addition, economical production of THA should be taken into consideration. In future, THA may be a viable alternative on bone grafting when clinical trials are completed.

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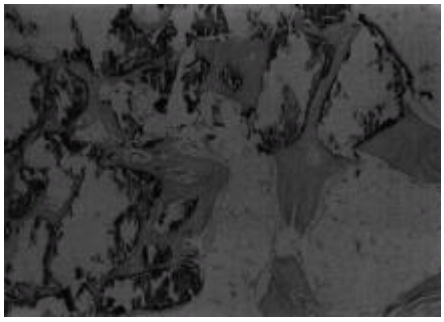

Figure 1: HEx40, bone growth around CHA

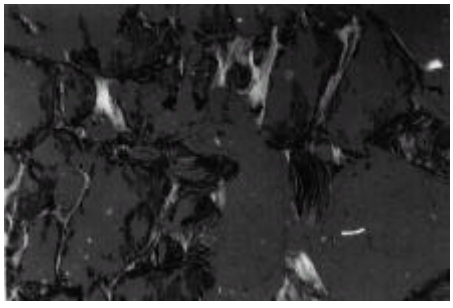

Figure 2: Same field with figure 1.
Organized arrangement of collagen fiber lameller pattern in new formed bone around particles, as viewed by polarization filters.

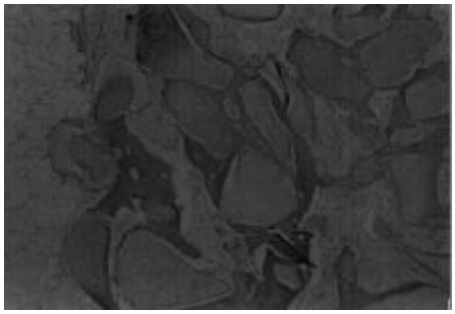

Figure 3: Hex40, bone growth around particle of DHA.

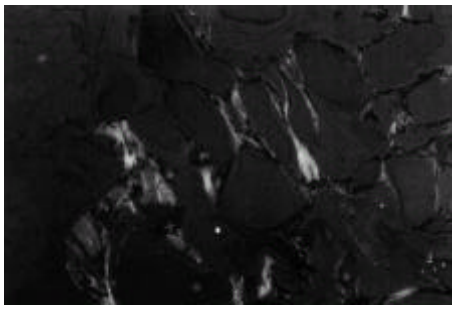

Figure 4: Same field with figure 3, as viewed by polarization filters.